

CLAIMS

1. A variant of a parent Fungamyl-like alpha-amylase, comprising an alteration at one or more regions selected from the group of:
- 5 Region 98-110,
Region 150-160,
Region 161-167,
Region 280-288,
Region 448-455,
10 Region 468-475.
- wherein (a) the alteration(s) are independently
- (i) an insertion of an amino acid downstream of the amino acid which occupies the position,
- (ii) a deletion of the amino acid which occupies the
- 15 position, or
- (iii) a substitution of the amino acid which occupies the position with a different amino acid,
- (b) the variant has alpha-amylase activity and (c) each region or position corresponds to a region position of the amino acid
- 20 sequence of the parent Fungamyl-like alpha-amylase having the amino acid sequence of SEQ ID NO: 2.
2. The variant of claim 1, wherein the variant is one or more of the following substitution: Q153S.
- 25 3. The variant of claims 1, which variant has improved thermostability and/or increased stability at acidic pH.
4. A DNA construct comprising a DNA sequence encoding an alpha-
- 30 amylase variant of any of claims 1-3.
5. A recombinant expression vector which carries a DNA construct according to claim 4.
- 35 6. A cell which is transformed with a DNA construct according to claim 4 or a vector according to claim 5.
7. A cell according to claim 6, wherein the cell is a microorganism, such as a bacterium or a fungus.

8. The cell according to claim 7, which is a protease deficient strain of *Aspergillus*, in particular *A. oryzae*.
9. A composition for producing high maltose syrup comprising an Fungamyl-like alpha-amylase variant of claims 1-3.
10. The composition of claim 9, further comprising beta-amylase activity.
11. A dough improving composition, comprising an alpha-amylase variant of any of claims 1-3.
12. A brewing composition comprising an alpha-amylase variant of any of claims 1-3.
13. The brewing composition of claim 12, further comprising one or more enzymes selected from the group of beta-amylase and isoamylase.
14. A composition for producing alcohol, comprising an alpha-amylase variant of any of claims 1-3.
15. A process of liquefying starch, wherein an alpha-amylase variant of claims 1-3 is used for treating starch.
16. A process of producing high maltose syrups, wherein an alpha-amylase variant of claims 1-3 is used for liquefying starch.
17. A brewing process, wherein an alpha-amylase variant of claims 1-3 is added during fermentation of wort.
18. An alcohol production process, wherein an alpha-amylase variant of claim 1-3 is used for liquefaction starch in a distillery mash.
19. A process, wherein a dough product comprising an alpha-amylase variant of claims 1-3 is baked.
20. Use of an alpha-amylase variant of any of claims 1-3 or a

composition of claim 9 for starch liquefaction.

21. Use of an alpha-amylase variant of any of claims 1-3 or a composition of claim 9 for producing alcohol.

5 22. Use of an alpha-amylase variant of any of claims 1-3 or a composition of claim 9 for brewing.

10 23. Use of an alpha-amylase variant of any of claims 1-3 or a composition of claim 9 for baking.

24. A method for generating an alpha-amylase variant of a parent Fungamyl-like alpha-amylase, which variant has increased thermostability, in particular at acidic pH relative to the parent, the method comprising:

- 15 (a) subjecting a DNA sequence encoding the parent Fungamyl-like alpha-amylase to random mutagenesis,
(b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and
20 (c) screening for host cells expressing a mutated alpha-amylase which has improved thermostability at acidic pH relative to the parent Fungamyl-like alpha-amylase.

25 25. Process for producing a maltose syrup comprising the steps of:

- 1) liquefying starch in the presence of an alpha-amylase, followed by
2) dextrinization the presence of a fungal alpha-amylase variant of claim 1-3;
30 3) recovery of the syrup; and optional purification.

26. Process for producing syrup, in particular maltose syrup, comprising the steps of:

- 1) liquefying starch at a temperature of 140-160°C at a pH of 4-
35 6, followed by
2) dextrinization at a temperature in the range from 60-95°C at a pH 4-6 in the presence of a fungal alpha-amylase variant of claims 1-3; and
3) recovery of the syrup; and optional purification.

27. The process of claim 26, wherein the liquefying starch is treated at a temperature of 65-85°C, in particular 70-80°C.
- 5 28. The process of claim 27, wherein an effective amount of glucoamylase is added in step 2).
29. Process for producing maltose syrup, comprising the steps of:
- 10 1) liquefying starch at a temperature of 95-110°C at a pH of 4-6 in the presence of a *Bacillus* alpha-amylase, followed by
- 2) dextrinization at a temperature in the range from 60-95°C at a pH 4-6 in the presence of a fungal alpha-amylase variant of claims 1-3; and
- 15 3) recovery of the syrup; and optional purification.
30. An immobilized variant of claims 1-3.

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